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The Yield and Thiamine, Riboflavin and Niacin Content of Alfalfa Hay as Related to Fertilizer Treatment of Soil

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THE YIELD AND THIAMINE, RIBOFLAVIN AND NIACIN CONTENT OF ALFALFA HAY AS RELATED TO FERTILIZER TREATMENT OF SOIL

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INTRODUCTION

Alfalfa has long been recognized as an outstanding hay plant in terms of both yield and feeding value. It is high in digestible protein, minerals, and vitamins. Because of its long life it can be economically produced. It is of exceptional value in maintaining soil fertility by helping to control erosion, by improving the physical condition of the soil, and by accumulating large amounts of nitrogen.

The great importance of alfalfa growing in the United States is attested by its rapid and continued expansion. It is one of the most important forage crops and is grown on a greater acreage than any other crop in the intermountain area.

From 1899 to 1919 the seventeen states west of Minnesota grew from 87 to 97 percent of the alfalfa in the United States. In 1927 these states grew 64 percent and in 1949, only 44 percent (8). It is said that if the acreage planted to legumes were to be increased to the extent advisable for soil conservation and a balanced agricultural economy, the annual need for alfalfa seed would increase to more than 100 million pounds (5).

About one-third of Utah's crop land and 38 percent of its irrigated land are planted to alfalfa. Livestock production is limited by the amount of forage that can be produced (10). An increase in the production of alfalfa would materially increase the number of livestock that could be fed. Perhaps more important is the quality of the hay. Modern health practices are concerned not only with the curative aspect of disease, but also with their prevention. They are primarily concerned with those conditions which contribute to the fullest realization of the inherited potentialities of the crop for growth, development and resistance to disease.

Investigations of the relationship between soil and nutrition have the important functions of showing how a greater quantity of better food can be provided for an increasing population. During most of man's agrarian existence he has been able to grow his food on the best soils. With rapidly increasing population, that day is passing and he must learn to use soils of a lower fertility level. For this reason basic information about the effect of soil characteristics on the nutritional quality of food should be obtained now in order that such problems can be solved intelligently when they arise. Of all the sciences and arts one of the greatest is adequate feeding of animals and man.

This study is an attempt to determine the effect of various fertilizers applied to different soils on the yield of alfalfa and on interrelationships with thiamine, riboflavin, and niacin content. These three vitamins of the B-complex series appear to be of unusual and fundamental importance as they are apparently indispensible to all forms of life.

REVIEW OF LITERATURE

Fertilizers

In reviewing the phosphate fertilizer research on alfalfa, only work in the western states, where the soil and climatic conditions are similar to that of Utah, will be considered.

An experiment conducted at the Arizona Station (16) on the University Farm near Tucson suggested that fertilization with phosphate materially increased the nutritional value of the hay by increasing production and phosphorus content.

Pittman (21) of the Utah Station has shown that there is a direct correlation between yields resulting from the application of manure or super phosphate and the phosphorus content of the hay; the greater the yields, the larger the percentage of phosphorus contained in the hay. From rather extensive cooperative field experiment trials with farmers scattered all over the state of Utah, Pittman and Burnham (22) using ammonium sulfate, treble super phosphate and potassium chloride as sources of nitrogen, phosphorus and potassium have shown that alfalfa failed to respond to potassium and nitrogen while it responded to superphosphate. This result was to be expected as alfalfa is a legume, and has the ability to obtain its nitrogen supply from the air. Apparently most Utah soils are well supplied with available potassium.

Results by Toevs et al. (31) from the Idaho Experiment Station showed that while 300 pounds of treble super phosphate to the acre applied to alfalfa gave the greatest yields, the most economical returns were obtained from applications of 75 and 125 pounds.

A rather recent study by Kinkle (14) at the New Mexico Station shows the effects of different rates and frequencies of application and different sources of phosphate on the yield of alfalfa. They show that less concentrated grades of superphosphate such as 16 to 20 percent have no advantage over more concentrated forms when applied on a phosphorus equivalent basis. They recommend application of superphosphate each year in the spring before active growth starts.

Vitamins

There is basic evidence of the effect of variation in mineral supply of the soil on the concentration of minerals in plants (18).

The work of Sheldon et al. (25) shows considerable differences in amino acids in lespedeza grown on five different types of Missouri soils. In another study they have shown that the levels of these amino acids in alfalfa were increased by treatment of the soil with manganese, boron, or a mixture of cobalt, copper and zinc.

Hauge (13) studied the effect of sulfur fertilization on amino acids and reported an increase in the methionine and cystine content.

In the case of thiamine, Schultz and coworkers (23) have shown that different samples of any one cereal can vary widely in the vitamin. He showed variation from 412 to 7.3 microgram per gram in samples of wheat. The work of Andrews (1) shows that both variety and environment influenced thiamine content. Kelley et al. (15) studied thiamine content of seven varieties of beans grown in two areas differing in soil type. They found variety differences but no differences were found due to locality.

Harris (12) reported that soil treatment with ammonium sulfate, complete mineral fertilizer or stable manure produced no significant effects on the thiamine content of wheat. Passmore and Sundararjan (19) grew wheat with and without applications of potassium nitrate, super phosphate and manure, and combinations of these without obtaining significant differences in thiamine. Whiteside and Jackson (32) reported that environment influences the thiamine content of Canadian hard red spring wheat. Hamner and coworkers (11) have analysed a number of varieties and selections of wheat grown in many parts of the United States. They found that the locations at which the wheat was grown produced differences among the varieties observed. Pfutser (23) described limited studies where nitrogen fertilization of barley grown in pots appeared to result in a higher content of thiamine. Hauge (13) found sulphur fertilization had no apparent effect upon thiamine and riboflavin in alfalfa.

Andrews et al. (1) in their studies of five varieties grown at four different places found that the values for riboflavin were almost the same, showing no environment differences. There were also no marked differences in varieties. Similarly in a study of soft and hard wheat no significant differences were found. These were in agreement with Commer and Straub (6), who also noted no significant differences in riboflavin content of soft and hard wheat.

Bondi and Mayer (4) found that plants grown in Palestine during the summer months contained more riboflavin than similar plants grown during the rainy winter season. Tepley et al. (26) studied the niacin content of wheat of differing varieties and sources. They reported variations in both cases. In recent studies Frey and Watson (7) made chemical studies on thiamine, riboflavin, niacin and pantothenic acid in different varieties and strains of oats. They showed variation in the niacin content from 4.4 microgram per gram of oats in Hurbon variety to 11.7 in C.I. 5298, while thiamine content ranged from 5.37 micrograms per gram in Mido to 9.69 in C.I. 5298. Riboflavin content in oats ranged from 1.05 microgram per gram to 1.89 in Worthy.

These and other studies on vitamin as reviewed by Maynard (17) have given inconclusive results. Perhaps a different approach to the problem is needed.

PLAN OF WORK

In 1947 and 1948 areas were established on farms in Cache, Duchesne, Emery, San Juan, Uintah and Utah Counties of Utah.

Each area was divided into plots of 18' X 75' except in Duchesne County where they were 15' X 90'. There were four replications of treated and untreated plots arranged as randomized blocks. In Utah and Cache Counties larger plots were established for feeding the hay to farm animals.

The fertilizers were added to the soil in the spring of 1947 or 1948 and the soil in the plots was seeded with certified Ranger alfalfa seed at that time.

Soil Analysis and Fertility Requirements

Use of chemical analysis of soils and plants for determining the fertilizer needs of soil dates back to 1840 with the publication of Leibig's epoch-making book "Organic Chemistry in Its Application of Agriculture and Physiology" (19). It is only recently, however, that much progress has been made. The following determinations were made on samples from each farm: calcium carbonate, available phosphorus, total soluble salts, organic matter and pH. The results are shown in table 1. The methods followed were from the soil laboratory manual by Thorne (30).

Alfalfa is a crop that has a high mineral requirement. A good crop removes calcium equivalent to 400 pounds pure limestone per acre annually from the soil. It frequently contains 2 percent potassium on the dry basis, large amounts of phosphorus, magnesium, and sulphur and also appreciable quantities of trace elements (3).

Practically all Utah soils contain ample to excessive amounts of calcium and most are well supplied with potassium. The soils analysed were rather low in available phosphorus. With this in mind the treatments in this project were made to include treble superphosphate alone, superphosphate and manure, and combinations of boron, cobalt, manganese, copper and zinc in small quantities, The role of these elements in successful alfalfa production has been established by the work of others (3) (27).

Chemical Studies on Thiamine, Riboflavin and Niacin

Vitamins are potent organic substances which occur in minute quantities in natural foodstuffs. No attempt will be made to describe their importance as it is well established.

Thiamine

Thiamine was determined by the thiochrome method as suggested by the Association of Vitamin Chemists (2). Thiamine on oxidation under suitable conditions gives the yellow-blue fluorescent pigment thiochrome, which fluoresces in ultra-violet light. A Coleman photofluorometer, model 12, was used. The content of vitamin was calculated by comparing fluorescence of samples with a standard U.S.P. thiamine.

Riboflavin and Niacin

Riboflavin and niacin were determined by microbiological assay using *Lactobacillus casei* and *Lactobacillus arabinosis 17-5*. The growth of the organisms was measured turbidemetrically at 650 millimicrons on a Junior Coleman spectrophotometer as suggested by the Association of Vitamin Chemists (2). The contents of the vitamins were estimated by comparing the values of the samples with a standard curve at different levels using standard U.S.P. riboflavin and niacin, respectively.

The methods used for the statistical analysis of the data are described by Snedecor (26). The data were analysed only with respect to variations in treatment and replication. No attempt was made to analyse the data with respect to various locations.

The least significant differences between treatment means were calculated. The coefficients of variation were also calculated. In some cases, particularly for the year 1949, where it was not possible to collect the yield date the approximate values were calculated and no attempt was made to analyse the data statistically.

Yield in Relation to Fertilizer Treatments

The yield data are presented in tables 2,3 and 4. The sources of variation are discussed below.

Treatments

There were highly significant differences in yield from the different treatments. The highest yields were obtained in the irrigated farms and the lowest on non-irrigated farms. This shows the importance of proper moisture for the successful production of alfalfa. The yields varied with the type and the amount of fertilizers added. In general, the highest yields were obtained from the plots treated with treble super phosphate plus cattle or chicken manure. The untreated plots invariably gave the lowest yield.

Cache County

For 1949 the best yields, 3.63 and 2.66 tons per acre, were obtained on plots treated with 400 pounds of treble super phosphate plus trace elements and 400 pounds of treble super phosphate plus copper sulfate, respectively. The lowest yield, 1.29 tons per acre, was obtained on the untreated plots. All plots except these treated with copper sulfate alone gave significantly higher yields than the untreated. In addition to the effect of phosphorus there was a significant effect of trace elements on yield.

In 1950 the best yields, 2.94 and 2.80 tons per acre, were obtained on plots treated with 400 pounds of treble super phosphate plus cattle manure and 400 pounds of treble super phosphate plus copper sulfate. The lowest yield, 1.06 tons per acre, was obtained on the untreated plots. No treatment in addition to 400 pounds of treble super phoaphate resulted in significant differences.

Duchesne County

In 1949 the best yields, 9.26 and 8.89 tons per acre, were obtained on plots treated with 400 pounds of treble super phoaphte plus cattle manure and cattle manure alone. The lowest yield, 7.55 tons per acre, was obtained on the untreated plots.

In 1950 the best yields, 4.35 and 4.24 tons per acre, were obtained on plots treated with 400 pounds of treble super phosphate plus trace elements and 400 pounds of treble super phosphate and cattle manure. The lowest yield 3.50 tons per acre, was obtained on the untreated plots. Plots treated with 400 pounds of super phosphate plus cattle manure and the same level of phosphorus plus trace elements showed significant differences as compared to untreated ones, but yields on these plots were not significantly better than on those treated with phosphate alone.

Uintah County

In 1949, on the Nielson farm, there were no significant differences in yield.

In 1950 also there were no significant differences found, except for phosphates. The highest yield was obtained on plots treated with 400 pounds of treble super phosphate plus trace elements while the lowest was obtained on the untreated ones.

Utah County

For 1949 the best yields, 3.95 and 3.71 tons per acre, were obtained on the plots treated with 400 pounds of super phosphate plus trace elements and 400 pounds of treble super phosphate plus 100 pounds of copper sulfate. The

Table 1. Chemical Analysis of Soil Samples Taken from Different Test Areas in Utah.

Name	Location (nearest town)	County	Depth in inches	pH of soil paste	Total soluble salts %	Organic matter %	$CaCO_3$ (lime) %	Available PO_4 p p m	Available K p p m
1. Evans	Lehi	Utah	0-6	7.9	0.053	2.8	6.2	4	-
			0-6	7.9	0.041	1.2	4.8	2	-
			12-18	8.0	0.042	-	16.0	-	-
			12-18	8.2	0.070	-	12.0	-	-
2. Nielson	Vernal	Uintah	0-8	8.0	0.070	2.1	8.0	4	85
			0-8	8.2	0.027	1.6	8.0	2	75
			18-24	8.0	0.035	-	7.0	-	-
			18-24	8.2	0.033	-	38.0	-	-
3. Taylor	Randlett	Uintah	0-8	8.1	0.024	1.2	16.0	4	55
			0-8	8.0	0.027	0.8	18.0	1	60
			18-24	8.2	0.020	0.4	17.0	0.6	50
4. Carhart	Monticello	San Juan	0-8	7.3	0.020	1.2	1.0	10	70
			0-8	7.6	0.020	1.2	1.0	13	110
			12-18	7.6	0.020	-	1.0	-	-
			24-32	7.9	0.034	1.2	2.0	-	-
5. Lymah	Monticello	San Juan	0-8	7.9	0.041	2.9	12.0	1	110
			0-8	7.8	0.041	3.8	14.0	2	120
			0-8	7.8	0.041	3.6	14.0	1	100
			0-8	7.7	0.028	3.7	19.0	1	140
			18-24	7.8	0.030	-	6.0	-	-
6. Wilson	Logan	Cache	0-8	8.0	0.045	4.0	18.0	2	320
			12-24	8.5	0.064	3.0	54.0	1	185
			30-36	8.1	0.076	1.3	42.0	0.5	180
7. Abbott	Duchesne	Duchesne	0-8	7.8	0.075	1.4	10.0	0	135
8. Tuttle	Castle Dale	Emery	0-6	7.7	0.110	2.3	22.0	2	60
			0-6	7.9	0.097	2.0	22.0	2	74
			0-6	7.8	0.110	1.9	21.0	2	81
			0-6	7.9	0.110	2.8	23.0	41	118
			0-6	7.9	0.084	1.7	20.0	3	88
			0-6	8.0	0.110	1.8	30.0	2	61

Table 2. Yield of Ranger Alfalfa Produced on Fertilized and Unfertilized Plots on different farms. 1949.

Name	Location	a 200 lbs tsp (1)	b 400 lbs tsp	c 400 lbs tsp plus 100 lbs $CuSO_4$	d 100 lbs $CuSO_4$	e 400 lbs tsp plus 8-10 tons cattle manure	f 8-10 tons cattle manure	g 400 lbs tsp plus trace elements (2)	h Control, no fertilizer added	
		(Treatments per acre - applied spring, 1948)								
		Values in tons per acre on air dry basis								
1. Wilson (unirrigated)	Cache									L.S.D.
	2nd crop	1.13	1.18	1.42	0.99	1.28	1.06	1.94	0.62	.16
	3rd crop	0.99	1.03	1.24	0.86	1.12	0.93	1.69	0.60	.056
	Total	2.12	2.21	2.66	1.85	2.40	1.99	3.63	1.29	.18
2. Abbott (irrigated)	Duchesne									
	1st crop(3)	3.05	3.21	3.27	2.60	3.42	3.28	3.14	2.79	-
	2nd crop	2.65	2.79	2.84	2.26	2.97	2.85	2.73	2.42	.32
	3rd crop(3)	2.56	2.70	2.75	2.19	2.87	2.76	2.64	2.34	-
	Total	8.26	8.70	8.86	7.05	9.26	8.89	8.51	7.55	-
3. Nielson (irrigated)	Uintah									
	1st crop(3)	2.40	2.46	2.42	2.28	2.61	2.39	2.36	2.46	-
	2nd crop	2.08	2.13	2.09	1.97	2.26	2.07	2.04	2.13	.26
	3rd crop(3)	2.02	2.06	2.03	1.91	2.19	2.01	1.98	2.06	-
	Total	6.50	6.65	6.54	6.16	7.06	6.47	6.38	6.65	-
4. Evans (irrigated)	Utah									
	1st crop(3)	1.18	1.12	1.37	0.73	1.46	1.13	1.31	0.63	-
	2nd crop(3)	1.02	0.97	1.19	0.63	1.26	0.98	1.14	0.55	-
	3rd crop	0.99	0.94	1.15	0.61	1.22	0.95	1.10	0.53	.39
	Total	3.19	3.03	3.71	1.97	3.94	3.06	3.55	1.71	-

(1) tsp = treble super phosphate; P_2O_5, 42%; CaO, 21.8% and some trace elements

(2) Borax, 50 lbs. cobaltous chloride, 35 lbs. manganese sulfate, 100 lbs; and zinc sulfate, 35 lbs. per acre.

(3) Approximate yield data were calculated.

Table 3. Yield of Ranger Alfalfa Produced on Fertilized and Unfertilized Plots on Different Farms, 1950.

Name	Location	a 200 lbs	b 400 lbs	c 400 lbs tsp plus 100 lbs $CuSO_4$	d 100 lbs $CuSO_4$	e 400 lbs tsp plus 8-10 tons cattle manure	f 8-10 tons cattle manure	g 400 lbs tsp plus trace elements (2)	h Control, no fertilizer added	
		(Treatments per acre - applied spring, 1948)								
		Values in tons per acre on air dry basis								
1. Wilson (unirrigated)	Cache									L.S.D.
	1st crop	0.74	0.75	1.03	0.36	1.11	0.54	1.00	0.42	0.32
	2nd crop	1.10	1.21	1.20	0.73	1.20	0.84	1.17	0.62	0.21
	3rd crop	0.36	0.52	0.57	0.00	0.63	0.15	0.47	0.00	0.20
	Total	2.20	2.48	2.80	1.09	2.94	1.53	2.64	1.06	0.60
2. Abbott (irrigated)	Duchesne									
	1st crop	2.91	3.09	2.81	2.77	3.26	2.68	3.51	2.65	0.62
	3rd crop	0.90	0.96	0.91	0.82	0.98	1.00	0.84	0.85	0.12
	Total	3.81	4.05	3.72	3.59	4.24	3.68	4.35	3.50	0.74
3. Neilson (irrigated)	Uintah 1st crop	1.19	1.30	1.19	1.05	1.47	1.14	1.50	1.09	0.41
4. Evans (irrigated)	Utah									
	1st crop	1.71	1.56	1.74	0.93	1.61	1.51	1.59	1.22	0.52
	2nd crop	2.26	2.48	2.21	1.88	2.17	2.23	2.40	1.96	0.55
	3rd crop	0.93	1.13	0.94	0.87	1.08	1.12	1.13	0.62	0.21
	Total	4.90	5.17	4.89	3.68	4.86	4.86	5.12	3.80	0.91

(1) tsp = treble super phosphate; P_2O_5, 42%; CaO 21.8% and some trace elements.
(2) Borax, 50 lbs; cobaltous chloride, 35 lbs; manganese sulfate, 100 lbs; and zinc sulfate, 35 lbs. per acre.

Table 4. Yield of Ranger Alfalfa Produced on Fertilized and Unfertilized Plots on Tuttle Farm, Emery County, Utah.

Name	Location	a 129 lbs tsp (1)	b 258 lbs tsp	c 516 lbs tsp	d 516 lbs tsp plus 100 lbs $CuSO_4$	e 516 lbs tsp plus 12-15 tons chicken manure	f 12-15 tons chicken manure	g 516 lbs tsp plus trace elements (2)	h Control, no fertilizer added	
		(Treatments per acre - applied spring, 1947)								
		Values in tons per acre on air dry basis								
Tuttle (irrigated)	Emery	year 1949								L.S.D.
	1st crop	1.11	1.72	2.45	2.53	3.19	2.84	2.58	0.50	0.39
	2nd crop	1.85	2.25	2.54	2.62	2.92	2.57	2.81	1.18	0.07
	Total	2.96	3.97	4.99	5.15	6.10	5.41	5.39	1.68	0.57
		year 1950								
	1st crop	1.01	1.49	2.43	2.30	3.21	2.63	2.42	0.68	0.37
	2nd crop	0.98	1.42	1.94	2.06	2.34	2.29	2.05	0.77	0.23
	Total	1.99	2.91	4.37	4.36	5.55	4.92	4.47	1.45	0.54

(1) tsp = treble super phosphate, available P_2O_5, 42%, CaO, 21.8% and trace elements.
(2) Borax, 48.4 lbs; cobaltous chloride, 24.2 lbs.; copper sulfate; 48.4 lbs; manganese sulfate, 98.6 lbs.

lowest yield, 1.71 tons per acre was obtained on the untreated plots. The primary effect was due to phosphorus.

In 1950 significant differences were obtained. All the yields except the ones from plots treated with 100 pounds of copper sulfate were significantly higher than yields from untreated plots. No treatment effect in addition to phosphorus showed significant differences.

Emery County

In Emery County the treatments applied were slightly different. Chicken manure was used instead of cattle manure and the levels of treble super phosphate used were somewhat varied.

In 1949 the best yield, 6.10 and 5.41 tons per acre were obtained on plots treated with 516 pounds of treble super phosphate anc chicken manure, and chicken manure alone, respectively. The lowest yield, 1.68 tons per acre was obtained on the untreated ones. All the treated plots showed significant differences as compared to untreated. Chicken manure increased the yield significantly above phosphorus alone.

In 1950, the best yield, 5.55 and 4.92 tons per acre was obtained on the plots treated with 516 pounds of treble super phosphate and chicken manure and chicken manure alone. The lowest yield was 1.45 tons per acre on the untreated ones. All yields from treated plots showed significant differences, as compared to those from untreated ones. Chicken manure alone gave significantly greater increases in yield than phosphate alone or in combination with any treatment except chicken manure.

Thiamine, Riboflavin and Niacin in Relation to Fertilizer Treatment

The analytical data of thiamine, riboflavin and niacin are presented in tables 8 and 9. The sources of variation are discussed below.

In most cases highly significant differences were obtained due to

Table 5. Reproducibility of Analytical Values for Thiamine in Alfalfa by Fluorometric Method

	Values in milligram per hundred grams				
Sample No.	Series 1	Series 2	Average of replications	Deviation from the mean	Percent deviation
250B	0.49	0.52	0.50	0.02	4.0
275B	0.48	0.48	0.48	0.0	0.0
300B	0.38	0.37	0.37	0.0	0.0
333B	0.37	0.42	0.39	0.03	7.8
382B	0.42	0.35	0.38	0.03	7.9
424B	0.34	0.39	0.36	0.03	8.3
477B	0.36	0.38	0.37	0.01	2.7
492B	0.45	0.47	0.46	0.01	2.18
500B	0.48	0.43	0.45	0.02	4.4
508B	0.58	0.48	0.53	0.04	7.5

Average percent deviation from the mean 4.38

Table 6. Reproducibility of Analytical Values for Riboflavin in Alfalfa by Microbiological Assay Method

Values in milligram per hundred grams					
Sample No.	Series 1	Series 2	Average of replications	Deviation from the mean	Percent deviation
250B	1.00	1.10	1.05	0.05	4.70
275B	1.12	1.23	1.17	0.06	5.10
300B	0.86	1.02	0.94	0.08	8.50
333B	1.29	1.31	1.30	0.01	0.40
382B	1.31	1.24	1.27	0.03	2.40
424B	0.98	1.10	0.04	0.06	5.80
477B	1.05	1.08	1.06	0.02	1.90
492B	1.33	1.42	1.37	0.05	3.60
500B	1.07	1.19	1.13	0.06	5.30
508B	1.11	1.04	1.07	0.03	2.80

Average percent deviation from the mean 4.10

Table 7. Reproducibility of Analytical Value for Niacin in Alfalfa by Microbiological Assay Method

	Values in milligram per hundred grams				
Sample No.	Series 1	Series 2	Average of replications	Deviation from the mean	Percent
205B	2.08	2.31	2.19	0.11	5.30
275B	2.33	2.61	2.47	0.14	6.00
300B	2.24	2.26	2.25	0.01	0.44
333B	2.20	2.17	2.18	0.02	0.88
282B	2.30	2.30	2.33	0.03	1.27
424B	2.37	2.31	2.34	0.03	1.26
477B	2.26	2.14	2.20	0.06	2.76
492B	2.62	2.48	2.55	0.08	3.04
500B	2.38	2.41	2.39	0.01	0.42
508B	2.40	2.69	2.54	0.14	5.84

Average percent deviation from the mean 2.76

different treatments. The results are given in more detail below:

Thiamine

Cache County

In the second crop the amount of thiamine varied from 0.32 to 0.40 milligram per hundred gram. Alfalfa from plots treated with 400 pounds of treble super phosphate plus trace elements contained significantly less thiamine than that from untreated plots.

In the third crop no significant differences were found.

Duchesne County

In the second crop significant differences were found with values ranging from 0.32 to 0.49 milligram per hundred gram. All the alfalfa except that from soil treated with 200 pounds of treble super phosphate and 8 to 10 tons of manure contained significantly less thiamine than alfalfa from untreated plots.

San Juan County

At Dove Creek, the values ranged from 0.40 to 0.48 milligram per hundred gram. The differences were approaching significance.

At Monticello, significant differences were found. The values ranged from 0.37 to 0.50 milligram per hundred gram. All plots except alfalfa from those treated with 400 pounds of treble super phosphate alone and 100 pounds of copper sulfate alone contained significantly less thiamine than that from untreated plots.

Uintah County

There were significant differences found in the first crop at Randlett with values ranging from 0.38 to 0.51 milligram per hundred gram. Alfalfa from plots treated with 400 pounds of treble super phosphate plus 100 pounds of copper sulfate and 400 pounds of treble super phosphate plus trace elements contained significantly less thiamine then that from untreated plots.

In the second crop at Vernal, there were no significant differences according to different treatments.

Utah County

There were significant differences found in the second crop. The values ranged from 0.29 to 0.49 milligram per hundred gram. Alfalfa from plots treated with 400 pounds of treble super phosphate plus trace elements, 8-10 tons of cattle manure and 400 pounds of treble super phosphate plus 100 pounds of copper sulfate contained significantly less thiamine while that from plots treated with 400 pounds of treble super phosphate plus 8 - 10 tons of cattle manure and 400 pounds of treble super phosphate alone contained significantly more than that from untreated plots.

In the third crop, no significant differences were found.

Emery County

There were no significant differences in the second crop. The values ranged from 0.36 to 0.42 milligram per hundred gram.

In the third crop significant differences were found. The values ranged from 0.42 to 0.59 milligram per hundred gram. Alfalfa from plots treated with 516 pounds of treble super phosphate plus trace elements contained significantly less thiamine while alfalfa from plots treated with 129 pounds of treble super phosphate alone contained significantly more than alfalfa from untreated plots.

<u>Riboflavin</u>

Cache County

In the second crop there were significant differences found. The values ranged from 0.89 to 1.28 milligram per hundred gram. Alfalfa from plots treated with 400 pounds of treble super phosphate plus trace elements contained significantly less riboflavin than hay from untreated plots.

Table 8. Treatment Means and Analysis of Variance of Thiamine, Riboflavin, [illegible] Niacin Content of Ranger Alfalfa Produced on Fertilized and Unfertilized Plots in Different Counties of Utah, 1949.

Treatment Means (values in milligram per hundred gram dry basis)

Treatments/acre (1)	Cache Petersboro Second Crop B_1	B_2	Niacin	Third Crop B_1	B_2	Niacin	Duchesne Duchesne Second Crop B_1	B_2	Niacin	San Juan Dove Creek First Crop B_1	B_2	Niacin
200 lbs tsp (2)	0.36	1.07	2.40	0.43	1.04	2.56	0.48	1.14	2.33	0.42	0.70	2.95
400 lbs tsp	0.32	1.03	2.70	0.39	1.14	2.69	0.39	1.70	[illegible]	0.43	0.68	2.57
400 lbs tsp plus 100 lbs $CuSO_4$	0.38	1.02	2.50	0.40	1.07	2.41	0.44	1.12	2.41	0.48	0.71	2.74
100 lbs $CuSO_4$	0.39	1.03	2.26	0.37	1.01	2.46	0.43	1.26	2.38	0.46	0.70	2.73
400 lbs tsp plus 8-10 tons cattle manure	0.40	1.03	2.38	0.43	1.07	2.48	0.43	1.16	2.47	[illegible]	.73	2.48
8-10 tons cattle manure	0.41	1.28	2.43	0.45	1.10	2.41	0.47	1.03	2.46	0.43	.68	2.71
400 lbs tsp plus trace elements(3)	0.32	0.89	2.27	0.44	1.08	2.45	0.43	[illegible]	2.37	0.47	.68	2.87
Control, no fertilizer added	0.37	1.15	2.40	0.43	1.08	2.65	0.49	1.34	2.15	[illegible]	.71	2.39
C.V. (4)	6.29	[illegible]	5.68	13.32	5.56	3.58	6.23	[illegible]	[illegible]	[illegible]	15.30	4.85
L.S.D. (5)	.0341	.1766	.197			.1356	.0408	.1568	.1778			.1896

Treatments/acre (1)	San Juan Monticello First Crop B_1	B_2	Niacin	Uintah Randlett First Crop B_1	B_2	Niacin	Uintah Vernal Second Crop B_1	B_2	Niacin	Utah Lehi Second Crop B_1	B_2	Niacin	Utah Lehi Third Crop B_1	B_2	Niacin
200 lbs tsp (2)	0.39	1.04	2.38	0.48	0.74	2.55	0.39	1.14	2.73	0.38	1.09	2.52	0.47	1.12	2.28
400 lbs tsp	0.45	1.01	2.35	0.51	0.74	2.59	0.41	1.20	2.36	0.49	0.99	2.50	0.43	1.22	2.49
400 lbs tsp plus 100 lbs $CuSO_4$	0.37	1.08	2.21	0.39	1.09	2.34	0.40	1.12	2.55	0.29	1.01	2.39	0.42	1.15	2.37
100 lbs $CuSO_4$	0.50	1.17	2.30	0.44	1.19	2.57	0.41	1.26	2.33	0.36	1.05	2.36	0.46	1.09	2.40
400 lbs tsp plus 8-10 tons cattle manure	0.42	0.99	2.46	0.50	1.04	2.50	0.41	1.16	2.29	0.44	0.67	2.42	0.45	1.24	2.47
8-10 tons cattle manure	0.39	1.19	2.29	0.46	0.96	2.44	0.41	1.03	2.40	0.33	1.02	2.37	0.44	1.15	2.30
400 lbs tsp plus trace elements(3)	0.40	1.05	2.31	0.38	1.06	2.53	0.40	1.22	2.13	0.34	1.05	2.33	0.49	1.14	2.46
Control, no fertilizer added	0.48	0.92	2.27	0.47	0.78	2.68	0.43	1.34	2.74	0.38	1.20	2.22	0.42	0.99	2.23
C.V. (4)	8.85	11.30	4.84	8.75	9.76	10.80	9.03	9.00	3.64	7.04	7.71	6.45	13.00	11.35	5.02
L.S.D. (5)	.0568			.058	.1344			.1566	.1333	.0437	.1184				

MEAN SQUARE (s^2) (6)

Sources of Variation (7)	df	Cache Second Crop B_1	B_2	Niacin	Cache Third Crop B_1	B_2	Niacin	Duchesne Second Crop B_1	B_2	Niacin	Dove Creek First Crop B_1	B_2	Niacin
Treatment	7	5.03**	52.2*	76.10**	2.83	5.9*	50.79	4.38**	34.3*	46.80*	2.82a.p	1.20	156.7
Replication	3	0.33	2.75	56.09*	3.36	2.8	6.98	1.50	0.56	8.10	1.90	16.0	21.85
Error	21	0.54	14.4	17.94	3.17	2.7	8.49	0.77	1.13	14.62	1.15	11.42	16.53
Total	31												

Sources of Variation (7)	Monticello First Crop B_1	B_2	Niacin	Randlett First Crop B_1	B_2	Niacin	Vernal Second Crop B_1	B_2	Niacin	Lehi Second Crop B_1	B_2	Niacin	Lehi Third Crop B_1	B_2	Niacin
Treatment	11.31**	32.36	23.91	10.26**	101.23**	43.01	0.68	34.33**	184.1**	16.03**	35.02**	37.20	1.93	23.87	35.50a.p
Replication	0.26	44.3*	2.52	0.47	21.99	23.71	0.71	5.64	8.07	0.43	14.26	4.90	1.14	1.62	10.27
Error	1.50	14.44	12.51	1.56	8.3	42.95	1.35	11.35	8.23	0.89	0.47	23.82	3.60	16.70	14.34
Total															

(1) Fertilizers applied spring, 1948
(2) Treble super phosphate; P_2O_5, 42%; CaO, 21.8% and some trace elements
(3) Borax, 50 lbs; cobaltous chloride, 35 lbs; manganese sulfate, 100 lbs zinc sulfate 35 lbs per acre.
(4) Coefficient of variation = $\frac{s}{\bar{x}}$ x 100.
(5) Least significant mean difference at 0.05 probability level.
(6) All values raised to 10^3
(7) Degree of freedom

* Statistically significant at 0.05 probability level.
** Statistically highly significant at 0.01 probability level
a.p. = approaching significant

Table 9. Treatment Means and Analysis of Variance of Thiamine, Riboflavin, and Niacin Content of Ranger Alfalfa Produced on Fertilized and Unfertilized Plots at Castle Dale, Emery County, Utah, 1949.

Values in milligram per hundred gram dry basis

	Treatment per acre (1)	129 lbs tsp (2)	258 lbs tsp	516 lbs tsp	516 lbs tsp plus $CuSO_4$	516 lbs tsp plus 12-15 chicken manure	12-15 tons chicken manure	516 lbs tsp plus trace elements (3)	Control, no fertilizer added	L.S.D. (4)
					Second Crop					
Treatment Means	Thiamine	0.35	0.41	0.36	0.37	0.42	0.41	0.39	0.40	
	Riboflavin	1.11	1.27	1.18	1.20	1.05	1.21	1.08	1.20	
	Niacin	2.40	2.42	2.12	2.27	2.51	2.14	2.24	2.47	.1325
					Third Crop					
	Thiamine	0.59	0.47	0.46	0.49	0.50	0.45	0.42	0.50	.0716
	Riboflavin	1.08	0.92	0.99	1.07	1.13	0.96	1.08	1.33	.1375
	Niacin	2.16	2.09	2.35	2.39	2.06	2.24	2.55	2.21	.1627

Sources of Variation (5)

		Treatment	Replication	Error	Total	C.V. (7)
	d.f.(6)	7	3	21	31	
Mean Squares (s^2)	Thiamin	2.77	4.85	2.19		12.00
	Riboflavin	22.89	29.61	16.98		11.60
	Niacin	89.26**	6.73	8.21		4.44
	Thiamine	10.26**	0.33	2.37		10.10
	Riboflavin	24.77*	1.27	8.74		8.90
	Niacin	108.92**	18.92	12.24		4.92

(1) Fertilizers applied spring 1947
(2) Treble super phosphate; P_2O_5 42%: CaO, 21.8% and some trace elements:
(3) Borax, 48.4 lbs; $CoCl_2$ 24.2 lbs. $CuSO_4$ 48.4 lbs. $MnSO_4$ 98.6 lbs per acre.
(4) Least significant mean difference at 0.05 probability level.
(5) Values raised to 10^3
(6) Degree of freedom
(7) Coefficient of variation = $\frac{S}{\bar{X}}$ x 100
* Statistically significant at 0.05 probability level
** Statistically significant at 0.01 prabability level

In the third crop, however, no significant differences were found. The values varied from 1.01 to 1.14 milligram per hundred gram.

Duchesne County

There were significant differences found in the second crop. The values ranged from 1.03 to 1.34 milligram per hundred gram. Except alfalfa from plots treated with 400 pounds of treble super phosphate and 100 pounds of copper sulfate alone, all others contained significantly less riboflavin than the alfalfa from untreated plots.

San Juan County

In the first crop at Dove Creek, no significant differences were found. The values ranged from 0.68 to 0.73 milligram per hundred gram.

In the first crop at Monticello, no significant differences were found. The values ranged from 0.92 to 1.19 milligram per hundred grams.

Uintah County

In the first crop at Randlett there were highly significant differences found with values ranging from 0.74 to 1.09 milligram per hundred grams. Alfalfa from all plots except those treated with 200 and 400 pounds of super phosphate contained significantly higher riboflavin thean alfalfa from untreated plots.

At Vernal, significant differences were found with values ranging from 1.03 to 1.34 milligrams per hundred grams. Alfalfa from plots treated with 400 pounds of treble super phosphate plus trace elements, 100 pounds of copper sulfate alone and 400 pounds of treble super phosphate contained significantly less riboflavin than alfalfa from untreated plots.

Utah County

In the second crop highly significant differences were found with values ranging from 0.87 to 1.20 milligrams per hundred grams. Alfalfa from all the plots contained significantly less riboflavin than that from untreated plots.

In the third crop, no significant differences were found. The values ranged from 0.99 to 1.24 milligrams per hundred grams.

Emery County

There were no significant differences found in the first crop and the values ranged from 1.05 to 1.27 milligrams per hundred grams.

In the third crop, significant differences were found and the values ranged from 0.92 to 1.13 milligrams per hundred grams. Alfalfa from all the plots contained significantly less riboflavin than that from untreated plots.

Niacin

Cache County

There were highly significant differences in the second crop with values ranging from 2.27 to 2.70 milligrams per hundred grams. Alfalfa from plots treated with 400 pounds of treble super phosphate contained significantly higher amounts of niacin than alfalfa from untreated plots.

In the third crop highly significant differences were also found. The values ranged from 2.41 to 2.69 milligrams per hundred grams. Alfalfa from plots treated with 200 pounds of treble super phosphate and 400 pounds of treble super phosphate contained significantly less niacin than alfalfa from untreated plots.

Duchesne County

In the second crop significant differences were found with values ranging from 2.15 to 2.47 milligrams per hundred grams. Alfalfa from all the plots except those treated with 400 pounds of treble super phosphate contained significantly higher niacin than alfalfa from untreated plots.

San Juan County

In the first crop at Dove Creek, significant differences were found. The values ranged from 2.39 to 2.95 milligram per hundred gram. Alfalfa from all plots except ones treated with 400 pounds of treble super phosphate plus cattle manure contained significantly higher niacin then the untreated alfalfa.

There were no significant differences found in the first crop at Monticello. The values ranged from 2.21 to 2.46 milligrams per hundred grams.

Uintah County

No significant differences were found in the first crop. The vaues ranged from 2.34 to 2.68 milligrams per hundred grams at Randlett.

At Vernal, highly significant differences were found. The values ranged from 2.13 to 2.74 milligrams per hundred grams. Alfalfa from all th plots except those treated with 200 pounds of treble super phosphate contained significantly less niacin that that from untreated plots.

Utah County

In the second crop, no significant differences were found. The values ranged from 2.23 to 2.49 milligrams per hundred grams.

In the third crop the differences were approaching significance. The values ranged from 2.23 to 2.49 milligrams per hundred grams. Alfalfa from all plots except those treated with 400 pounds of treble super phosphate plus cattle manure and the same level of phosphate plus copper sulfate and 200 pounds of treble superphosphate alone contained significantly more niacin than alfalfa grown on untreated soil.

Emery County

In the second crop highly significant differences were found. The values ranged from 2.12 to 2.51 milligrams per hundred grams. All alfalfa samples except those from plots treated with 129 pounds of treble super phosphate and 258 pounds of treble super phosphate and 516 pounds of treble super phosphate plus chicken manure contained significantly less niacin than samples from untreated plots.

In the third crop highly significant differences were found. The values ranged from 2.06 to 2.55 milligrams per hundred grams. Alfalfa from plots treated with 516 pounds of treble super phosphate plus copper sulfate and the same level of phosphate plus trace elements contained significantly more niacin than untreated alfalfa.

DISCUSSION

In almost all cases alfalfa responded to phosphate fertilizer. However, variations were found due to natural fertility of the soil. Phosphorus applied in combination with manure gave the best yields except in Cache County for the year 1949 and in Duchesne County for 1950, where the best yields were obtained from the plots treated with 400 pounds of treble super phosphate plus trace elements. Previous results obtained (10) for the year 1948 show that in Emery County at Castle Dale plots treated with the 400-pound phosphate treatment gave the best yields. In addition to this, chemical analysis of the hays showed higher amounts of trace elements as compared with hays grown on the plots given other treatments (11).

If the comparison is made between plots treated with treble super phosphate and treble super phosphate plus copper sulfate, significant differences were found due to the presence of copper sulfate only at two locations in Cache and Utah Counties, and only for the year 1949. No such differences were found for the year 1950 anywhere. Of all the test areas, the Tuttle farm in Emery County showed the best response to fertilizer treatments for the year 1949 and 1950. Next to this, came the Wilson farm in Cache County and on the Abbott farm in Duchesne County.

Three levels of phosphorus were used and at least for the two years a residual effect of the fertilizer was observed. This phase of work is still under study and further results will show the length of time for which the residual effect is obtained.

Copper sulfate applied alone had no value as a fertilizer.

Irrigation always favored good growth of alfalfa. This shows the importance of proper moisture for successful production of alfalfa.

The vitamin contents were usually either statistically significant at the 0.05 or 0.01 probability level with respect to treatment. The effect of any one particular fertilizer treatment was not consistent from crop to crop, county to county or location to location in the same county. In rare cases differences in replications had statistical significance even at 0.05 probability level.

These results show that different soils and different fertilizer treatments modify the thiamine, riboflavin and niacin content of Ranger alfalfa hay. It is very difficult to find any correlation between any treatment and the concentration of a particular vitamin. This points to the extremely complicated mechanism of soil and plant relationships in biosynthesis of bioorganic compounds.

SUMMARY

(1) An experiment was carried out at seven locations in Utah to determine the effect of fertilizers on the yield and content of thiamine, riboflavin and niacin of alfalfa. The field experiment was a randomized split plot design, with four replications.

(2) Soil samples were taken for chemical analysis. The soils were found to be low in available phosphorus, low in organic matter, medium in soluble salts and pH.

(3) Due to the difference in the fertility level and irrigation practices between individual farms it is not possible to make specific recommendations in regard to fertilizer requirements. Generally speaking where soils are low in available phosphorus heavy infrequent applications may be preferable.

(4) Phosphorus was the primary fertilizer element needed for increased production.

(5) Trace elements significantly increased the yield above phosphate treatment alone only in Cache County in 1949. In 1950 trace elements exerted no significant effects on yield.

(6) Copper sulfate alone did not have any value as a fertilizer. In one case significantly higher yields were obtained with copper sulfate and super phosphate than with super phosphate alone.

(7) In the chemical phase of this work, 352 different samples of Ranger alfalfa grown on fertilized and unfertilized plots in Cache, Duchesne, Emery, San Juan, Uintah and Utah counties in the year 1949 were analyzed for thiamine, riboflavin and niacin.

(8) In general, statistically significant differences were found in the content of the three vitamins in Ranger alfalfa of the same crop in the

same location due to fertilizer treatments.

(9) The effect of any one fertilizer treatment was not consistent from crop to crop, county to county, or location to location in the same county.

(10) The lowest average concentration of thiamine, 0.29 milligram per hundred grams was found in Utah County while the highest 0.59 milligram per hundred gram was found in the third crop of Emery County.

(11) The lowest average concentration of riboflavin, 0.68 milligram per hundred gram was found in the first crop of San Juan County. The second crop at Vernal, Uintah County, showed the highest value of 1.34 milligrams per hundred grams.

(12) The lowest average concentration of niacin, 2.06 milligrams per hundred grams was found on the Tuttle farm, in Emery County and the highest average concentration of 2.95 was found in San Juan County.

(13) Out of eleven different crops analysed for thiamine, riboflavin and niacin significant differences were found in seven. In addition to fertilizer treatment, physiological development of the plant, sampling technique, techniques of analysis and climatic factors may have contributed to the variations observed.

BIBLIOGRAPHY

1. Andrews, J. S., H. M. Boyd and D. E. Terry. The riboflavin content of cereal grains and bread and its distribution in products of wheat milling. Cereal chem. 19:55 1942.

2. Association of Vitamin Chemists. Methods of Vitamin Assay. Interscience Publishers, Inc., New York 1947.

3. Bear, F. E. and Arthur Wallace. Alfalfa, its mineral requirements and chemical composition. Agr. Exp. Sta. Bull. 748. 1950.

4. Bondi, A. and H. Hayar. The riboflavin content of poultry feeding stuffs. J. Agri. Sci. 36:6 . 1946.

5. Carlson, J. W., et al. Growing alfalfa for seed in Utah. Utah Agr. Exp. Sta. Cir. 125 1950.

6. Comer, R. T. and G. J. Straub. The thiamine and riboflavin content of wheat and corn. Cereal chem. 18:671 1941.

7. Frey, K. J. and G. I. Watson. Chemical studies on oats, I Thiamine, riboflavin and pantothenic acid. Agron. Jour. 42:434 1950.

8. Graber, L. F. A century of alfalfa culture in America. Agron. Jour. 42: 525 1950.

9. Greenwood, D. A., D. W. Pittman et al. The interrelationship between composition of the soil and plants and the nutrition of animals and man. Utah Agr. Exp. Sta. Proj. 273. Logan, Utah

10. Greenwood, D. A. and D. W. Pittman. Fertilizers affect composition as well as yield of alfalfa. Farm Home Sci. Utah Agr. Exp. Sta. 11 (1): 12 1950.

11. Hamner, K. C., C. B. Lyon and C. I. Hanner. Effect of mineral nutrition on the ascorbic and acid content of the plant. Bot. Gaz. 103:589 1942.

12. Harris, L. J. Note on the thiamine potency of wheat as influenced by soil treatments. Jour. Agr. Sci. 24:410 1934.

13. Hauge, S. M., et al. Chemical composition and nutritive value of plants in relation to sulfur fertility of soil. Ind. Agr. Exp. Sta. 62nd Ann Rept. 1948-49.

14. Hinkle, D. A. Use of phosphate fertilizer on alfalfa. N. Mex. Agr. Exp. Sta. Bull. 289 1942.

15. Kelley, E., K. S. Dietrich, and T. Porter. Thiamine content of eight varieties of beans grown in two locations in Michigan. Food Res. 5:253 1940.

16. McGeorge, W. T. and J. F. Breazeale. Fertilization of alfalfa on alkaline calcarious soils. Ariz. Agr. Exp. Sta. Bull. 154 1936.

17. Maynard, L. A., and K. C. Beeson. Some causes in variation in the content of plants grown for food. Nutri. Abst. and Rev. 13:155 1943.

18. Mrak, E. M., and G. F. Stewart (Edited) Advances in Food Research. 1:291 Academic Press, Inc. New York 1948.

19. Moulton, F. R. Liebig and After Liebig - a Century of Progress in Agricultural Chemistry. Am. Assoc. Adv. Sci. Washington, D. C. 1942.

20. Passmore, R and A. R. Sundavarrajan. Ind. J. Med Res. 29:90 1941 (Nutri. Abs. and Rev. 13:155 1943).

21. Pittman, D. W. Effect of manure and phosphate fertilizer on the yield and composition of alfalfa hay. Utah Agr. Exp. Sta. Bull. 247 1934.

22. Pittman, D. W., and C. Burnham. Observations on the use of commercial fertilizers on the arid soils of Utah. Utah Agr. Exp. Sta. Bull. 233 1932.

23. Pftutzer et al. Biochem. 2 298:137 1938 (Nutr. Abs. and Rev. 13:155 1943.

24. Shultz, A. S., L. Atkin and C. N. Fry. A primary study of the vitamin B_1 content of American cereals. Cereal chem. 18:106 1941.

25. Sheldon, L. V. et al. Diversity of amino acid in legumes according to soil fertility. Science 108: 426 1948.

26. Snedecor, G. W. Statistical Methods. Iowa State College Press, Ames, Iowa. 1946.

27. Stiles, W. Trace Elements in Plants and Animal. The Macmillan Company New York, N. Y. 1948.

28. Tepley, L. J., F. M. Strog, and C. A. Elvehjem. The distribution of nicotinic acid in foods. J. of Nutrition 23:417 1942.

29. Thorne, D. W. and H. P. Peterson. Irrigated Soils, Their Fertility and Management. The Blakiston Company, Philadelphia 1949.

30. Thorne, D. W. Laboratory Manual for Soil Analysis. Utah State Agr. College, Logan 1949.

31. Toeves, J. L. and G. O. Baker. Progress report of phosphate and other fertilizer investigations at the Aberdeen Branch Exp. Sta., Univ. of Idaho. Idaho Agr. Exp. Sta. Bull. 230 1939.

32. Whiteside, A. D. O. and S. H. Jackson. The thiamine content of Canadian hard red spring wheat varieties. Cereal chem. 20: 542 1943.